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TRANSLATION NO. 1619

[redacted]

DATE: 13-18 JAN 1964

AD835157

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17

RE-EXAMINATION OF SOME PHYSIOLOGICAL CHARACTERISTICS OF XANTHOMONAS ORYZAE(UYEDA ET ISHIYAMA) DOWSON.

Annals of the Phytopathological Society of Japan, 29, No. 1, 13-18(Jan 1964).

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Introduction

With respect to the physiological characteristics of Zanthomonas oryzae(Uyeda and Ishiyama) Dowson, the causative organism of the bacterial blight of the rice plant, there is a detailed study by Ishiyama(3). There are similar studies by Kuwazuka(7)(8) and Fang(2), but with respect to certain tests concerning the physiological characteristics of the pathogen, such as the milk coagulation test, or the reduction of dyes, there has not always been an agreement in the results. With changes in the variety of rice and the method of cultivation in recent years, the disease has spread to the Tohoku region, and depending on the location of the infected area, the existence of a number of different strains of the disease has been discovered(5)(18)(20). Despite these studies, little is still known of the relation between the physiological characteristics of the bacteria and the variation in pathogenicity among different strains, or the differences in affinity between the main strain and the isolates. The present experiment was performed in order to clarify some of these problems.

During the course of carrying out this experiment, we had the cooperation of Toshihiko Kusaba(Tottori Prefectural Agricultural Experimental Station), Minoru Watanabe(Tokyo Agricultural and Industrial College), and Tetsu Wakimoto (National Institute of Agricultural Sciences), to whom the authors are indebted.

1. Specimens and method of experiment

From among some 118 isolates obtained from rice plant leaves infected with bacterial rice blight disease received from all over the country, some 20 typical isolates (Table 1), based on differences in pathogenicity, were selected for testing with respect to nitrate and methylene blue reduction, decomposition of carbohydrates, and protein and amino acid reactions. The selected isolates were transplanted from the preserved culture medium to the following medium and allowed to grow for two days at 27°C.

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Potato broth(Potato 300g. distilled water 1000ml)	
$Ca(NO_3)_2.4H_2O$	0.5g
$Na_2HPO_4.12H_2O$	2.0g
Peptone	5.0g
Cane sugar	15.0g
Agar-agar	20.0g
pH	6.8-7.0

Table 1. History of isolates.

	No. of isolate	Location of collection	Date of separation
Isolates of strong pathogeniciy	N 5808	Miyazaki-ken Sendai-shi Iwakiri-daigahara	15 Aug 1958
	N 5824	Tokyo-to Tama-gun Yuki-mura	6 Oct 1958
	N 5828	Fukui-ken Mikata-machi Bessho	1 Sep 1958
	N 5840	Shiga-ken Urabe-gun Ando-machi	30 Oct 1958
	N 5851	Okayama-ken Tsuyama-shi	3 Sep 1958
	N 5858	Yamaguchi-ken Toyoura-gun Kikugawa-machi	13 Oct 1958
	N 5866	Kochi-ken Nagaoka-gun Okatoyo-mura	27 Oct 1958
	N 5877	Saga-ken Koshiro-gun Mikazuki-mura	1 Sep 1958
	N 5879	Kumamoto-shi Akitsu-machi	28 Aug 1958
	N 5901	Yamagata-ken Akumi-gun Matsuyama-machi	13 Oct 1959
	Kofunya	Fukuoka-ken Kofunya	Kyushu Agr. Exp. Sta.strain.

Isolates of weak pathogenicity	N 5603	Kofu-shi Shimokawara-machi	4 Sep 1956
	N 5609	Tottori-ken Iwami-gun Ooiwamura	7 Oct 1956
	N 5617	Kumamoto-ken Yabe-machi	20 Sep 1956
	N 5707	Tokushima-ken Ootani	19 Sep 1957
	N 5802	Akita-ken Oomagari-shi	22 Aug 1958
	N 5818	Kanagawa-ken Ashie-gun Matsuda-machi	15 Sep 1958
	N 5835	Nagano-shi Agr. Exp. Station	17 Oct 1958
	N 5882	Nagasaki-ken Nerihaya-shi Sakaeda	17 Oct 1958
	Tokai		Strain preserved at Tokaikinki Agr. Exp. Station.

The experiments for studying the physiological characteristics of the isolates were as follows (9) (16) (22).

Reduction of nitrate: Isolates were cultivated at 27°C in a peptone-water medium containing 0.1% potassium nitrate. The degree of reduction was tested three times, on the third, fifth, and the seventh day, by the color reaction to Gries' reagent.

Reduction of methylene-blue: The isolates were cultivated in bouillon (about 10ml). On the first and the fourth days, a drop of saturated methylene-blue solution was added, the solution agitated, and upon quieting of the solution, the degree of reduction was tested by means of bleaching of the color.

Generation of ammonia: The isolates were cultivated in a peptone-water solution. Three times, i.e., on the third, the fifth, and the seventh days, Nessler's reagent was added to test for ammonia formation.

Formation of Indole: The isolates were cultivated in a peptone-water medium. Three times, i.e., on the second, the fourth, and the seventh days, Kovac's reagent was added to test for formation of indole by a red color reaction.

Generation of hydrogen sulfide: After transplanting the isolates to the medium previously described, the culture was maintained at 27°C in a test tube with a piece of lead paper hanging into the test tube and held in place by a cotton plug. Blackening of the lead paper was checked four times between the first and the seventh days.

Liquefaction of gelatin: The medium here was 20% gelatin added to bouillon with the gelatin medium forming the upper layer. After intermittent sterilization in an autoclave, the isolate was transplanted to this medium and maintained at 12-18°C temperature. At seven day intervals, liquefaction of the gelatin was checked. This experiment was performed on three different occasions, i.e., from 10 December 1959, from 2 March 1960, and from 9 November 1960.

Coagulation of milk: A fixed amount (10ml) of fresh skimmed milk was introduced into a test tube and after three days of intermittent sterilization in a Koch sterilizer, the isolates were transplanted to the medium and maintained at 27°C. During the 14-day culture period, the samples were checked for degree of coagulation, digestion, coloration, and viscosity.

Change in litmus milk: The isolates were transplanted to a medium of skimmed milk to which some litmus had been added. Cultivating at a temperature of 27°C, the samples were checked

for bleaching of or for any change in the color of the litmus.

Diastase action: The isolates were transplanted to a medium consisting of 0.2% soluble starch in bouillon. Maintaining the culture at 27°C, saccharification of the starch was checked on the second, the fifth, and the tenth days by titration of potassium iodide tincture.

Formation of acids from saccharides and the higher alcohols: With peptone-agar as the basic culture medium, and with an addition of 0.2% B.T.B., 1% saccharides and higher alcohols forming the upper stratum, stab and streak slant cultures were made and maintained at 27°C. The generation of gas and acid formation were checked six times during the period of culture, i.e., on the first, second, fifth, seventh, tenth, and the fourteenth days.

Serological reaction: An antiserum prepared with a stock culture, 22-R-2, of X.oryzae as the antigen, this latter isolate having been obtained from another experiment, was inactivated by a thirty minute treatment at 57°C, and diluted twenty times with an isotonic solution. Taking 0.5ml of this into a test tube, 1.0ml and 5.0ml of a new suspension (about 10^8) of the isolates were added, the mixtures then being lightly agitated and maintained at 28°C. Coagulation reaction was checked after one hour and also after 17 hours.

2. Experimental results

Reduction of nitrates was not observed with any of the isolates. Reduction of indicators, namely methylene-blue, was not observable after one day, but such action was clearly indicated after the fourth day. There was only a slight difference between the isolates, the action being weaker with the isolates of weaker pathogenicity, such as N 5707, N 5802, and N 5882. Ammonia production occurred slowly, not observable after two days, but with some production occurring after four days, and a more clear indication of production with all of the isolates after seven days of culture. According to the color reaction to Nessler's reagent, there was some variation among the isolates with respect to the degree of ammonia production. There was no indole production with any of the isolates. With respect to hydrogen sulfide, there was considerable production starting from around the second day, and after four days, the majority of the lead paper strips were blackened. With respect to this test, there was considerable variation among the isolates, gas production being pronounced with Kofunya, N 5808, N 5603, N 5617, and N 5609, but quite weak in the case of N 5840. From these results, no relationship could be established between the pathogenicity, the date of collection, the date of separation, and the classification of the isolates of the X.oryzae virus according to their susceptibility.

The liquefaction of gelatin occurred very slowly. For instance, with the culture at room temperature (12-18°C), it just started after seven days, and after 3 to 4 weeks, liquefaction was generally well progressed with a dish-shaped to bag-shaped contour. The liquefaction in the case of the strongly pathogenic isolates, i.e., N 5808, N 5824, N 5828, and N 5840, was stratiform (Table 2). The liquefaction of gelatin was studied for some forty isolates of both strong and weak pathogenicities, the results being shown in Table 3.

Table 2. Physiological characteristics of X.oryzae.(a)

	(1) 菌株名	(2) 硝酸塩の還元	(3) 色素の還元	(4) アンモニアの発生	(5) インドールの発生	(6) 硫化水素の発生	(7) ゼラチンの溶解	(8) 牛乳の凝固	(9) 牛乳の消化	(10) リトマスミルクの青変	(11) ディスターゼ作用	(12) ガスの発生
(13) 病原性の強い菌株	N5808	−	+	+	−	++	++(b)	−	−	+	−	−
	N5824	−	+	+	−	+	++(b)	−	−	+	−	−
	N5828	−	+	+	−	++	++(b)	−	−	+	−	−
	N5840	−	+	+	−	±	++(b)	−	−	+	−	−
	N5851	−	+	+	−	+	+	−	−	+	−	−
	N5858	−	+	+	−	+	+	−	−	+	−	−
	N5866	−	+	+	−	+	++	−	−	+	−	−
	N5877	−	+	+	−	+	+	−	−	+	−	−
	N5879	−	+	+	−	+	+	−	−	+	−	−
	N5901	−	+	+	−	+	++	−	−	+	−	−
	紅粉屋 (15)	−	+	+	−	++	+	−	−	+	−	−
(14) 病原性の弱い菌株	N5603	−	+	+	−	++	+	−	−	+	−	−
	N5609	−	+	±	−	++	+	−	−	+	−	−
	N5617	−	+	+	−	++	+	−	−	+	−	−
	N5707	−	±	+	−	+	++	−	−	+	−	−
	N5802	−	±	+	−	+	+	−	−	+	−	−
	N5818	−	+	+	−	+	++	−	−	+	−	−
	N5835	−	+	±	−	+	+	−	−	+	−	−
	N5882	−	±	+	−	+	+	−	−	+	−	−
	東海 (16)	−	+	+	−	+	+	−	−	+	−	−

(a)・反応の強度は＋の多少で表示し，反応のみられないものは－とした。
(b)・ゼラチンを層状に液化した菌株

Legend: (a) /Footnote a/ The degree of the reaction is indicated by the number of + signs, a negative result being indicated by -.
(b) Isolates which caused stratiform liquefaction of gelatin.
(1) Name of Isolate. (2) Nitrate reduction.
(3) Indicator reduction. (4) Ammonia production.
(5) Indole production. (6) Production of hydrogen sulfide.
(7) Liquefaction of gelatin. (8) Coagulation of milk.
(9) Digestion of milk. (10) Litmus milk reaction.
(11) Diastase action. (12) Gas production.
(13) Isolates with strong pathogenicity.
(14) Isolates with weak pathogenicity.
(15) Kofunya. (16) Tokai.

Table 3. Relation between pathogenicity and liquefaction of gelatin.(a)

	(1) 菌株名	(2) 液界の組床 (3) 第1回実験	(4) 第2回実験		(1) 菌株名	(2) 液界の組床 (3) 第1回実験	(4) 第2回実験
	N 5701	＋	＋[b]		N 5603	＋	－
	N 5708	＋	＋		N 5604	＋	±
(5)	N 5809	＋	＋	(6)	N 5609	＋	－
病	N 5819	＋	＋	病	N 5612	＋	－
	N 5823	＋	＋		N 5617	±	－
原	N 5824	＋[b]	＋[b]	原	N 5618	＋	±
	N 5834	＋[b]			N 5803	＋	－
性	N 5839			性	N 5810	＋	±
	N 5840	＋	＋		N 5821	±	－
の	N 5844	＋	＋	の	N 5826	＋	－
	N 5866	＋			N 5827	＋	－
強	N 5873	＋		弱	N 5829	＋	－
	N 5874	＋			N 5846	±	－
い	N 5901	＋	＋	い	N 5854	＋	－
	N 5902	＋	＋		N 5853	＋	－
菌	N 5903	＋	＋	菌	N 5855	±	－
	N 5904	＋	＋		N 5861	＋	－
株	N 5905	＋	＋	株	N 5862	±	－
	紅 梅 屋	＋	±		N 5872	－	－
	新 庄	＋	±		東 海	＋	－

[a] 第1回実験は第2表に準ず。第2回実験は 10% ゼラチン培地で室温 6°C～28°C, 平均 13.9°C で 1960 年 11 月 9 日 培養, 1961 年 1 月 29 日 調査。

[b] ゼラチンを層状に液化した菌株。

(7) 菌株は第1表以外のものも用いたが表には省いた。

Legend: (a) Footnote Table 3 The first series of experiments is based on the data in Table 2. The second series of experiments involves a medium with 10% gelatin, room temperature 6-28°C, average temperature 13.9°C, culture started 9 November 1960 and checked 29 January 1961.
(b) Footnote Table 3 Isolates which caused stratiform liquefaction of gelatin.
(1) Name of Isolate. (2) Degree of liquefaction.
(3) First series of experiments. (4) Second series of experiments.
(5) Isolates with strong pathogenicity.
(6) Isolates with weak pathogenicity.
(7) Note that isolates other than those listed in Table 1 were also used, but data on them have been omitted.

It can be seen from the table that considerable liquefaction occurred with the isolates with strong pathogenicity, but at the same time, there were a few which displayed weak liquefaction.

There was no coagulation or digestion of milk, but there was a blue color reaction in the litmus milk test with all of the isolates.

Results were negative with respect to diastase action and the production of gas.

The action of the bacteria with respect to decomposition of saccharides and higher alcohols and the formation of acids is as shown in Table 4. As can be seen from this table, xylose, arabinose, glucose, levulose, galactose, mannose, and sucrose (monosaccharides and disaccharides) are decomposed to form acids. Among other saccharides, rhamnose, maltose, and lactose are not decomposed, while arabinose was decomposed by seven isolates, viz., N 5840, N 5877, N 5603, N 5609, N 5617, N 5818, and Tokai. With respect to saccharides of higher order than the trisaccharides and the higher alcohols, i.e., raffinose, dextrin, starch, inulin, glycerol, mannitol, sorbitol, and salicin, none of these were decomposed.

With respect to an antiserum with an antigen consisting of a streptomycin-resistant isolate of X.oryzae 22-R-2, all of the isolates had a positive agglutination reaction, there being however some variation among the isolates with respect to the speed and the intensity of the reaction.

Table 4. Saccharide decomposition by X.oryzae.

	菌株名 (1)	xylose	arabinose	rhamnose	glucose	levulose	galactose	mannose	sucrose	maltose	lactose
(2) 病原性の強い菌株	N5808	++*	−	−	++	+	++	+	++	−	−
	N5824	++	−	−	++	++	++	++	++	−	−
	N5828	++	−	−	++	++	++	++	++	−	−
	N5840	++	++	−	++	++	++	++	++	−	−
	N5851	++	−	−	++	++	++	++	++	−	−
	N5858	++	−	−	++	++	++	+	++	−	−
	N5866	++	−	−	++	++	++	+	++	−	−
	N5877	++	++	−	++	++	++	+	++	−	−
	N5879	++	−	−	++	++	++	++	++	−	−
	N5901	++	−	−	++	++	++	+	++	−	−
	紅粉屋	++	−	−	++	++	++	++	++	−	−
(3) 病原性の弱い菌株	N5603	++	++	−	++	++	++	++	++	−	−
	N5609	++	++	−	++	++	++	+	++	−	−
	N5617	++	++	−	++	++	++	++	++	−	−
	N5707	++	−	−	++	++	++	++	++	−	−
	N5802	++	−	−	++	++	++	++	++	−	−
	N5818	++	++	−	++	++	++	++	++	−	−
	N5835	++	−	−	++	++	++	++	++	−	−
	N5882	++	−	−	++	++	++	+	++	−	−
	東 春	++	++	−	++	++	++	++	++	−	−

* ++反応の明らかなもの，+反応の弱いもの，−反応を示さないもの。

Legend: (a) /Footnote a/ ++ means clear reaction, + weak reaction, - no reaction.

(1) Name of isolate. (2) Isolates with strong pathogenicity. (3) Isolates with weak pathogenicity.

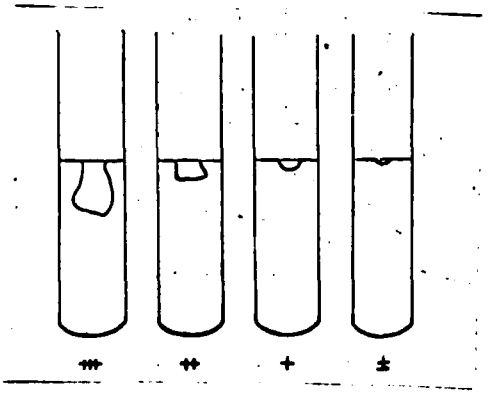

Figure 1. Criteria for liquefaction of gelatin.

Table 5. Serological reaction of isolates.

	(1) 菌株名	(2) 処理1時間後 A*	(2) 処理1時間後 B	(3) 処理17時間後 A	(3) 処理17時間後 B		(1) 菌株名	(2) 処理1時間後 A	(2) 処理1時間後 B	(3) 処理17時間後 A	(3) 処理17時間後 B
(4) 病原性の強い菌株	N 5701	+	+	++	++	(5) 病原性の弱い菌株	N 5604	±	−	++	+
	N 5708	++	±	++	++		N 5612	++	++	++	++
	N 5809	++	+	++	++		N 5617	++	++	++	++
	N 5823	±	−	++	++		N 5803	++	±	++	+
	N 5824	±	−	+	+		N 5826	++	±	++	±
	N 5840	++	++	++	++		N 5827	++	±	++	++
	N 5901	++	++	++	++		N 5829	±	−	++	+
	N 5902	±	−	++	++		N 5846	++	±	++	++
	N 5903	++	++	++	++		N 5853	++	++	++	++
	N 5904	++	++	++	++		N 5854	++	−	++	++
	N 5905	++	+	++	++		N 5855	++	±	++	+
	N 5868	±	−	++	++		N 5835	++	++	++	++
	紅粉屋	±	−	++	+		東春	±	−	++	++

* 反応の程度は +, − で表示した。
† Aは抗原 1 ml, Bは 5 ml 注加。
(6) 第1表以外の菌株も用いたが来歴は省いた。

Legend: (a) Footnote* Degree of reaction indicated by +, − signs. (b) Footnote† A has 1ml of antigen, B has 5ml of antigen injected.
(1) Name o isolate. (2) 1 hour after treatment.
(3) 17 hours after treatment. (4) Isolates with strong pathogenicity. (5) Isolates with weak pathogenicity.
(6) Isolates other than listed in Table 1 were used, but their descriptions have been omitted.

According to Table 5, the agglutination reaction was slow in the cases of N 5823, N 5824, N 5902, N 5868, Kofunya, N 5604, N 5829, and Tokai, particularly so in the case of N 5824. Despite this result, no relation could be ascertained between the pathogenicity and the previously described physiological characteristics.

3. Discussion

Research in the field of the systems and classification of plant pathogens has become gradually active in recent years. Okabe and Goto (12) (13) (14) have shown that in the case of the bacterial wilt (Pseud.solanacearum) affecting the eggplant family, there are variations in the reaction to lactose, and also dextrose and mannitol. They have also attempted a classification based on virial affinity, saccharide decomposibility,

and chromogenesis, and they present a discussion on the parasitic nature of the disease.

With respect to the rice blight caused by X.oryzae, Yoshimura et al (21) classified 76 isolates, obtained from the Hokuriku district, into five bacterial types, A, B, C, D, and E by means of the bacteriophages of the pathogen. He found that in the Hokuriku district, the A-type virus was most widely distributed, followed by type B, with types C and E being relatively rare. He did not find any definite relation with respect to the pathogenicity of the bacteria, but it was noted that the type C bacteria were of somewhat weaker pathogenicity. Wakimoto (18) has also experimented with a variety of isolates, and he has not found any relation involving pathogenicity. Isaka (5), investigating some 30 isolates from Fukui Prefecture, obtained similar results.

In Table 2 are shown the results of experimentation with 20 isolates selected from some 118 strains collected from various parts of the country and maintained by the National Institute of Agricultural Sciences, the experimentation being primarily concerned with clarification of the physiological characteristics of the isolates, the discovery of some relation between such characteristics and pathogenicity, and examination of the basis of classification of the pathogens. The present results disagree, as can be seen from the table, with the physiological characteristics determined by Ishiyama (3) and Kuwazuka (8) in many respects. For instance, Ishiyama (3) had no reduction of methylene-blue, no liquefaction of gelatin, no ammonia production, little production of hydrogen sulfide, but digestion of milk. Our results indicated reduction of methylene-blue, very slow but sure liquefaction of gelatin, abundant production of hydrogen sulfide, but no digestion of milk. On the other hand, we find that Pordesimo (15) proposes a change in the classification of X.oryzae, based on various physiological and culture characteristics, to X.translucens var.oryzicola. It is clear that the bacteria used by Pordesimo differs from the rice blight being considered here, his organism probably being X.oryzicola reported by Fang and his coworkers (2).

With respect to the decomposition of saccharides, Ishiyama (3) has experimented with a few saccharides, while more recently, Mukai et al (10) have conducted a detailed investigation with thirty isolates. Mukai found that xylose, arabinose, glucose, levulose, galactose, mannose, and sucrose were decomposed but that raffinose, dextrin, starch, inulin, glycerol, mannitol, sorbitol, and salicin could not be utilized. There was also no relation involving the pathogenicity. These results were in agreement with the data in our Table 4. It is seen that arabinose

is not decomposed by some isolates, while other isolates could only weakly utilize levulose and mannose, but such slight variations in characteristics are unacceptable for the purpose of classification of the isolates. With respect to other physiological characteristics, there were variations among the isolates with respect to the production of hydrogen sulfide, the liquefaction of gelatin, the production of ammonia, and the reduction of indicators. The variation in the first two characteristics was generally quite evident (Table 2). It is seen that there is a fairly close connection between the degree of liquefaction of gelatin and the pathogenicity, that is to say, isolates with stronger pathogenicity tended to liquify gelatin easier, but then on the other hand, there are also exceptions to this rule, such that further investigation on the basic nature of this relation is desirable.

With respect to serological differences among the isolates, there seemed to be some variation in the speed and intensity of the agglutination reaction, this reaction always being positive. These results agree with those obtained by Kuwazuka (7) and Fang et al (2). No relation could be observed between pathogenicity and the extent of the reaction, or between pathogenicity and the reaction.

4. Summary

(1) Twenty typical isolates of different pathogenicities were selected from some 118 isolates taken from leaves infected with rice blight disease in various sectors of the country, and experiments were conducted on the physiological characteristics of X.oryzae, the causative organism.

(2) None of the isolates tested reduced nitrates, but they reduced methylene-blue. These isolates generated ammonia, did not produce indole, produced hydrogen sulfide, and slowly liquefied gelatin, but did not coagulate or digest milk. The litmus milk had a blue color reaction. These results differed with those obtained by Ishiyama (3) with respect to methylene-blue reduction, the degree of hydrogen sulfide production, ammonia production, liquefaction of gelatin, and the digestion of milk.

(3) There was a fairly close relation between the liquefaction of gelatin and the pathogenicity.

(4) With respect to decomposition of saccharides and higher alcohols, there was decomposition of xylose, arabinose, glucose, levulose, galactose, mannose, and sucrose with resultant acid formation, but there was no utilization of rhamnose, maltose, lactose, raffinose, dextrin, starch, inulin, glycerol, mannitol,

sorbitol, or salicin. Some of the isolates did not decompose arabinose.

(5) There was some variation in the speed and intensity of the aglutination reaction with the isolate. The reaction with the anti-X.oryzae (22-R-2)-serum was positive. (Received 18 April 1963).

References:

(1) Bergey's Manual of Determinative Bacteriology. (1957). The Williams Wilkins Co.

(2) Fang, C.T., H.C.Ren, T.Y.Chen, H.C.Faan, and S.C.Wu, Acta Phytopath.Sinica 3(2), 99-124(1957)

(3) Ishiyama, S., Bull.Nat.Inst.Agr.Sciences, 45, 232-261(1922).

(4) Ishimaya, S. and H.Mukai, Shokubutsubyogensaikinshi (Journal of Plant Diseases). 1944. Meibundo Publ.Co., Tokyo.

(5) Isaka, M.et al, Report Hokuriku Disease and Pest Research Society, 9, 30-32(1961).

(6) Kusaba, T. et al, Intermed.Research Rpt.Nat'l Inst. Agr.Sciences, 13, 32-43(1960).

(7) Kuwazuka, K.Ann.Phytopathol.Soc.Japan, 2,(2), 169(1928).

(8) Kawazuka, K. Nogyo(Agriculture), 634, 14-27(1933).

(9) Manual of method for pure culture study of bacteria. Edited by the Committee on Bacteriological technique of the Society of American Bacteriologist.

(10) Mukai, H. et al, Ann.Phytopathol.Soc.Japan, 25(5), 229 (1960).

(11) Nishida, F., Nojizappo(Miscellaneous News in Farming), 68-75(1908).

(12) Okabe, T. and M.Goto, Shizuoka University Agricultural Research Report 2, 64-93(1952).

(13) Op.cit., 2, 94-114(1952).

(14) Op.cit., 3, 52-80(1952).

(15) Pordesimo, A.N., The Philippine Agriculturist, 42(4), 115-128(1958).

(16) Takimoto, K. Biseibutsugaku oyobi shokubutsubryorigakujikkenho (Microbiology and Plant Pathology, Experimental Methods in), Yokendo Publ.Co., Tokyo (1952).

(17) Wakimoto, T., Kyushu Univ.Agricultural Bulletin, 14(4), 485-493 (1954)

(18) Wakimoto, S., Ann.Phytopath.Soc.Japan, 25(4), 193-198 (1960).

(19) Yoshimura, S., Report Hokuriku Disease and Pest Research Society, 6, 62-85 (1958).

(20) Op.cit., 7, 43-52 (1959).

(21) Op.cit., 8, 21-24 (1960).

(22) Saikingakujisshuteiyo (Practical Manual on Bacteriology), Edited by Alumni Assn.Nat'l Inst.Infect.Diseases, Maruzen Bookstore, Tokyo (1956).

Resume

1. Physiological characteristics of Xanthomonas oryzae, the causal organism of the bacterial blight of rice plant, have been studied by several authors, but there are some discrepancies in their results with regard to certain characteristics such as reaction in milk and reduction of methylene-blue. The present research was carried out to make these points clear and to elucidate the relationship between the physiological properties and pathogenicities of the bacterium. For this purpose, 20 isolates of different pathogenicities were selected from 118 isolates which had been collected from various districts of Japan.

2. All the isolates used in this experiment reduced methylene-blue, but not nitrate; produced ammonia and hydrogen sulfide, but not indole; gas production and diastase activity were negative; liquefied gelatin gradually; did not coagulate or digest milk; and discolored Litmus milk into blue. Thus the result differs from Ishiyama's original description as to the reduction of methylene-blue, the liquefaction of gelatin, and the production of hydrogen sulfide.

3. Isolates more active in liquefying gelatin were, with some exception, more pathogenic than those with less liquefying power, suggesting that there might be an intimate relationship

between the liquefying power and the pathogenicity. There were some immunogenetical differences among the isolates in terms of the rate and strength of agglutination reaction, although the reaction was positive in all the isolates. The differences in the reaction, however, were correlated with the differences in the pathogenicity of the isolates.

4. The bacteria in culture were capable of decomposing xylose, arabinose, glucose, levulose, galactose, mannose, and sucrose, producing acids; and incapable of utilizing rhamnose, dextrin, starch, inulin, glycerol, mannitol, sorbitol, and salicin. Some of the isolates could not utilize arabinose.

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